Ion Composition Elucidation (ICE): a High Resolution Mass Spectrometric Technique for Identifying Compounds in Complex Mixtures

Andrew H. Grange and G. Wayne Sovocool

U.S. EPA, Office of Research and Development, National Exposure Research Laboratory, Environmental Sciences Division, Environmental Chemistry Branch, P.O. Box 93478, Las Vegas, NV 89193-3478

When tentatively identifying compounds in complex mixtures using mass spectral libraries, multiple matches or no plausible matches due to a high level of chemical noise or interferences can occur. Worse vet, most analytes are not in the libraries. In each case, Ion Composition Elucidation (ICE) provides a means for identifying compounds. This poster illustrates an example of each problem and its solution.

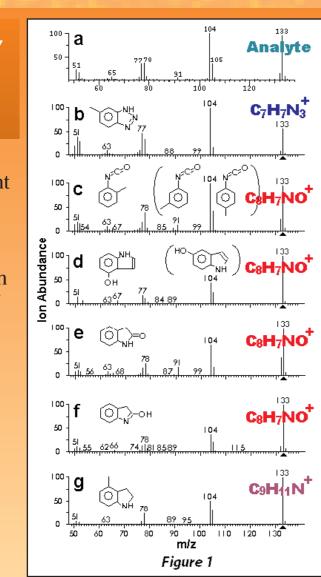
Ion Composition Elucidation (ICE) to Provide Additional Mass Spectral Data

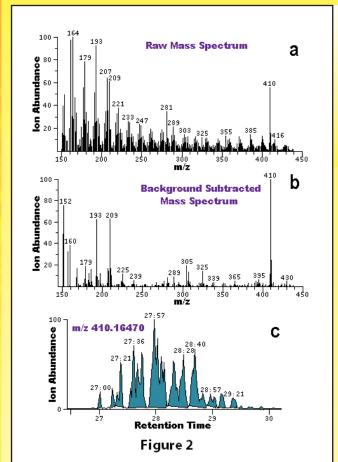
ICE was developed by the Environmental Chemistry Branch of the National Exposure Research Laboratory of the Office of Research and Development of the U.S. Environmental Protection Agency to help identify compounds in environmental samples. ICE has two facets, data acquisition using Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD)[1,2] and automated data interpretation using a Profile Generation Model (PGM).[3]

Three Compound Identification Problems

1. Multiple Plausible Library Matches

The mass spectrum in Figure 1a is a background-subtracted mass spectrum for a compound in an extract of 12 L of effluent from a tertiary waste water treatment plant. Figures 1b-g are NIST library matches over the same mass range. The isomers in parenthesis in Figure 1 also had similar NIST mass spectra. The compound that provided the mass spectrum was present in the extract at an ultra-trace level. Chemical noise, coelution of compounds in the complex extract, and septum and column bleed components generally result in background-subtracted mass spectra containing extraneous ions or lacking low-abundance ions expected from the analyte. Hence, none of the NIST library matches can be ruled out without additional data.





2. High Chemical Noise and Few Mass Peaks for Library Matching

In Figures 2a and b raw and background-subtracted mass spectra

are shown of a compound that produced a prominent ion at m/z 410. Because no other ion chromatograms were superimposible with that of the m/z 410 ion, only this ion could be associated with the compound with certainty. The NIST library returned 205 hits when a molecular weight of 410 was entered. Of these, numerous mass spectra provided m/z 410 as the most prominent ion over this mass range. Figure 2c, the ion chromatogram for m/z 410.16470 acquired with 10,000 resolution, suggests an isomeric series of compounds. Here again, additional data were required to reach tentative identifications for the compounds in Figure 2c.

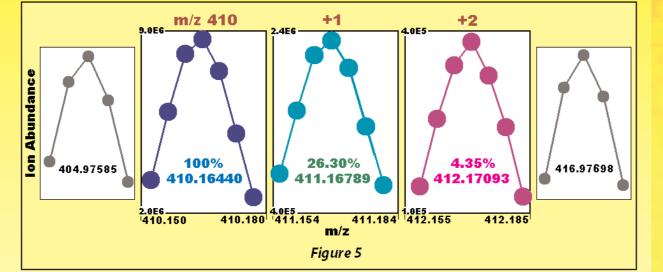
MPPSIRD

Using the selected ion recording (SIR) mode, multiple m/z ratios are monitored across full or partial mass peak profiles for up to three analyte ions and two calibration ions. The chromatographic peak areas delineated by the ion chromatograms are plotted to provide the profiles. The insets in Figure 4a are ion chromatograms corresponding to the maxima in calibrant ion and analyte ion profiles. For ever-present calibrant ions, a simulated chromatographic peak is created by the two baseline excursions in the ion chromatogram, which are induced by reversing a lens polarity in the ion source for 5 s.

Figures 4a-c illustrate the multi-step process for determining an ion composition. First, (Figure 4a) a survey was made of a wide mass range (1600 ppm) about the analyte profile using 3000 resolution and a mass increment between the m/z ratios of 100 ppm to ensure the correct profile was further studied and to obtain a coarse estimate of its exact mass. Two profiles were observed. The lighter mass profile was due to an ever-present compound, the calibrant PFK, while the higher mass profile was plotted from ion chromatograms that displayed a chromatographic peak for the analyte. Next, a full

mass peak profile was obtained with 10,000 resolution (Figure 4b) using the estimated exact mass from Figure 4a as the center mass in the SIR descriptor. A narrower mass range was viewed (200 ppm) using a mass increment of 10 ppm. The weighted average of the top 10 points provided an exact mass accurate to within 6 ppm. Finally, because this mass and error limit did not correspond to a unique composition, profiles for both this apparent molecular ion (M) and the ions containing higher +1 isotopes such as ¹³C, ¹⁵N, ¹⁷O, and ²H were obtained (Figure 4c), again using 10,000 resolution and 10 ppm mass increments. The exact mass of the +1 profile and its abundance relative to M were used to determine the correct composition. The relative abundance is the ratio of the sums of the seven areas used to plot each partial profile x 100%.

In Figure 5 are shown well-defined, interference-free partial profiles for the m/z 410 ion and its +1 and +2profiles. The +2 profile is also obtained routinely to provide two additional measurements. Note that in place of a single exact mass, three exact masses and two relative abundances are available for comparison to calculated values for different ion compositions.



Shown in Figure 6 are the partial profiles for the m/z 213 ion and its +1 and +2 profiles. Again, interference-free profiles provided three exact masses and two relative abundances.

PGM

A profile generation model calculated Gaussian distributions to construct composite profiles arising from ions containing +1 or +2 isotopes and automatically compared measured and calculated exact masses and relative abundances.

Table 1

m/z 133.06408 ± 6 ppm Resolution: 10,000 Elements Considered: CHNOFPSSi

		Mass [Defects	Relative Abundance				
# RDB Composition		133 +1		%+1 (Range %+1)				
1 0.0	C2 H6 N6 F	.06380	.06413 X	4.52 (3.90-5.14) X				
2 1.5	C3 H10 N4 P	.06431	.06588 X	4.96 (4.27-5.64) X				
3 6.0	C7 H7 N3 ✓	.06400	.06661	8.99 (7.75-10.23)				
Expe	rimental Values:	.06408	.06705	9.39				

Table 1 shows the output provided after the two exact masses and relative abundance in Figure 4c were entered. Only the three compositions listed had calculated exact masses that agreed with the measured exact mass within the error limit of its determination. Each "X" next to an entry indicated the measured and calculated values were inconsistent and the composition in that row was rejected. Only the third composition, $C_7H_7N_3$, passed all three comparisons. Thus, it is the correct composition of the m/z 133 ion.

Tentative Identification

Determination of C₇H₇N₃ as the correct composition rejected compounds with the other two compositions, C₈H₇NO and C₉H₁₁N. Only the isomer in Figure 1b had this composition, 5-methyl-1Hbenzotriazole. It is a component of x-ray film developer. ICE narrowed the number of possible compounds from nine to one, and commercial use considerations also led us to purchase a single compound for confirmation. However, this compound provided a very similar mass spectrum, but a different retention time. The analyte in the extract might be 6-methyl-1H-benzotriazole, but no standard was obtainable. This analyte is still being investigated.

m/z 410.16440 ± 1.2 ppm Resolution: 10,000 Elements Considered: C(1/3)* H N O P S *At least 1/3 of the mass was assumed to be from C atoms

Table 2

	At least 1/3 of the mass was assumed to be from C atoms								
#	Composition	M	(Error)	M+1	(Error)	M+2	(Error)	%M+1	M+2
57	$C_{22}H_{25}N_3O_3P$.16335	(-2.6)	.16651	(-3.4)	.16932	(-3.9)	25.93	3.76
56	C ₂₄ H ₂₇ O ₄ P ✓	.16470	(+0.7)	.16810	(+0.5)	.17104	(+0.3)	27.24	4.36
60	$C_{25}H_{23}N_4P$.16603	(+4.0)	.16913	(+3.0)	.17222	(+3.1)	29.88	4.24
61	$C_{26}H_{22}N_2O_3$.16304	(-3.3)	.16629	(-3.9)	.16926	(-4.1)	29.57	4.86
Exp'l. Values		.16440		.16789		.17093		26.30	4.35
61	$C_{26}H_{22}N_2O_3$.16304	,	.16629	,	.16926	,	29.57	4.86

Table 2 is a modified PGM output that lists the exact mass differences between the measured and calculated values for the m/z 410 ion and its +1 and +2 partial profiles. Four compositions passed all five comparisons. However, the data were acquired and integrated across a time window that included all of the isomers observed in Figure 2c. With at least 25 isomers present, the 6 ppm error limit was divided by $\sqrt{25}$ to provide 1.2 ppm as the error limit based on 25 determinations of the exact masses. This lower error limit rejected 3 of the 4 compositions based on each of the three exact masses, leaving $C_{24}H_{27}O_4P$ as the correct composition.

Tentative

Only one NIST library match, tri-xylyl-phosphate, was found for C₂₄H₂₇O₄P. The methyl groups could be at any two of the five available positions on each of three rings, which gives rise to a great many possible isomers and

Tentative Identification

fonyl)benzothiazole.* Its structure was consistent with

A chemical catalog contained only one compound

with the composition, $C_8H_7NO_2S_2$, 2-(methylsul-

the observed fragment ions. It was purchased and

found to have the same retention time and similar

ions as seen in Figure 3c. *

Hence, the compound was

instance of very good luck.

identified. This was an

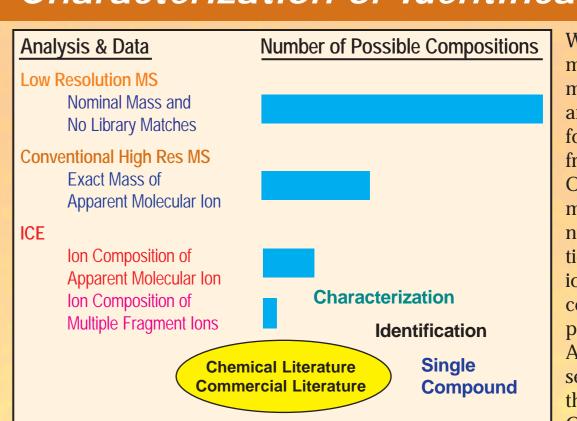
relative abundances for the m/z 134, 150, and 213

Identification

accounts for the large number observed.

<u>Application</u>	Reference	Poster at www.epa.gov/nerlesd1/chemistry/ecb-posters2.htm
Characterization of a Superfund Site	LC-GC, 1996, 14, 478-486	─ ✓
Identification of Well Pollutants —	Rap. Comm. MS, 1998, 12, 1161-1169 —	─ ✓
Confirmation of Synthetic Products Probe Introduction	with —J. AOAC Int'l., 1999, 82, 1443-1457 ——	─ ✓
Deconvolution of CI Isotopic Pattern	ns ————	─ ✓
Determination of Ion Compositions without Mass Calibrants	———Rap. Comm. MS, 1999, 13 , 673-686——	✓
Hemoglobin Adduct Mass Measuren	nent —	 ✓
Forensic Chemistry Applications —	Int'l. J. Environ. Foren., 2001, 2, 61-74	

Characterization or Identification?



Applications of ICE

When poor-quality, low resolution mass spectra provide only a nominal mass for an apparent molecular ion, and no credible library matches are found, the ion could be produced from a great many compounds. Obtaining an exact mass for the molecular ion greatly reduces this number, although multiple compositions remain possible for higher-mass ions. Using ICE to determine the ion composition reduces the number of possible compounds considerably. And determining the compositions of several fragment ions further limits the number of possible isomers.[4] Compounds are seldom identified

with this technique alone, but greatly limiting the number of possible isomers makes literature searches to take this final step feasible. When ICE greatly limits the number of possible isomers, an unidentified compound has been characterized. When a literature search provides candidates, standards can be purchased to complete the compound's identification.

MPPSIRD Advantages

MPPSIRD provides important advantages relative to full scanning or conventional SIR when only profile maxima are monitored.

Speed - 31 m/z ratios are observed during each 1 s cycle. The data required is collected as chromatographic peaks elute.

Sensitivity - the 100-fold increase in sensitivity realized by SIR is retained.

Selectivity - higher sensitivity can be traded for higher resolution to better discriminate against mass

Stability - recalibration against the lock mass each cycle avoids apparent resolution loss when data are acquired using volatilization from a probe or infusion with electrospray ionization.

Exact mass measurement - an exact mass rather than a nominal mass is obtained.

Relative abundance measurement - relative abundances of +1 and +2 profiles are obtained with greater accuracy than by full scanning.

Resolution measurement - the mass divided by the width of a monoisotopic profile at 5% of maximum provides the mass resolution when the compound elutes.

Interference detection - a significant interference uplifts the lower or higher-mass tail.

References

- 1. Grange, A.H.; Donnelly, J.R.; Brumley, W.C.; Billets, S.; Sovocool, G.W. Anal. Chem., 1994, 66, 4416-4421.
- 2. Grange, A.H.; Donnelly, J.R.; Brumley, W.C.; Sovocool, G.W. Anal. Chem., 1996, 68, 553-560.
- 3. Grange, A.H.; Brumley, W.C. J. Amer. Soc. for Mass Spectrom., 1997, 8, 170-182
- 4. Grange, A.H.; Sovocool, G.W.; Donnelly, J.R.; Genicola, F.A.; Gurka, D.F. Rapid Commun. Mass Spec., 1998, 12,

Notice: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded this research and approved this poster. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

3. Analyte Not in the Library

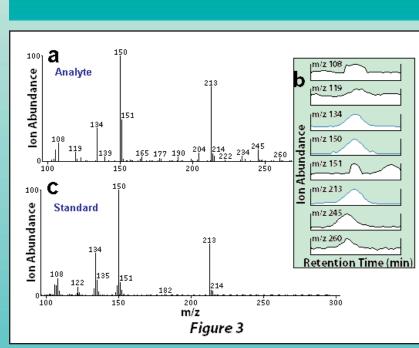


Figure 3a is a background-subtracted mass spectrum with an apparent molecular ion at m/z 213. In Figure 3b, only the ion chromatograms of the m/z 134 and 150 ions are superimposible with that of the 213 ion. No plausible matches were obtained when these three ions were entered into the NIST library program. Yet again, additional data were required to tentatively identify this compound.

Table 3

 $m/z = 212.99183 \pm 6 ppm$ Resolution: 10,000 Elements Considered: CHNOPFSSi

ı	#	RDB Range	Composition	M	M+1	M+2	%M+1(%M+1 Range)	%M+2 (%M+2 Range)
ı	90	11.5	C ₁₁ H ₂ N ₂ FS	.99227	.99516	.98865	13.37 (11.46 - 15.33) X	4.68 (3.70 - 5.59) X
ı	91	10.5	C ₁₂ H ₆ PS	.99278	.99596	.98904	13.48 (10.87 - 15.99) X	4.59 (3.56 - 5.54) X
ı	92	11.5	C ₁₂ H ₃ OPF	.99055	.99392	.99682 X	14.02 (11.51 - 16.72) X	0.37 (0.00 - 0.69) X
ı	93	15.5	C ₁₃ HN ₂ Si	.99090	.99315 X	.98981 X	17.72 (13.76 - 21.61) X	4.37 (3.15 - 5.25) X
	83	6.0 8.0	C ₈ H ₇ NO ₂ S ₂ ✓	.99182	.99451	.98796	10.64 (8.92 - 12.37)	9.22 (7.70 - 10.60)
1								

After entering the three exact masses and two relative abundances from Figure 6 into the PGM, an output table was obtained. Table 3 is the lower portion of that table. Only one composition, C₈H₇NO₂S₂, was consistent with the five measured values.